COMPARATIVE CHEMO-THERAPEUTICAL STUDY OF ATOXYL AND TRYPANOCIDES

PART II

BY

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(Received for publication 9 December, 1908)

Our previous work[1] on the chemo-therapeutics of Atoxyl has led us to the conclusion that a combination *in vitro* takes place between proteins, and Atoxyl mono-acetylated Atoxyl and mono-benzoylated Atoxyl respectively; whilst, on the other hand, such a combination does not occur between proteins, and sodium arsenate acetyl-benzoyl Atoxyl and sodium-p-hydroxy-phenyl-arsenate. This work has been continued by injecting the above-mentioned drugs into experimental animals; these reacting in an analogous way to the serum-proteins *in vitro*, with only one exception—acetyl-benzoyl Atoxyl—which combined with the serum proteins *in vivo*. This reaction, however, was only to be expected, as the organism saponifies the acetyl group, and the resulting benzoyl Atoxyl acts in the same way as mono-benzoyl Atoxyl *in vitro*.

Technique.—Rabbits were injected for several months, twice weekly, with Atoxyl, Sodium arsenate, acetylated and benzoylated Atoxyl, Benzoyl-acetyl Atoxyl, and Sodium-hydroxyl-phenyl-arsenate. Usually 1 c.c. of 1 per cent. solution of the drug was injected.

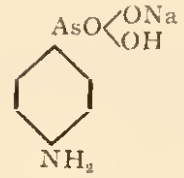
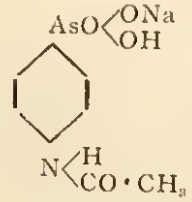
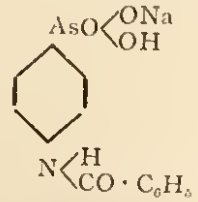
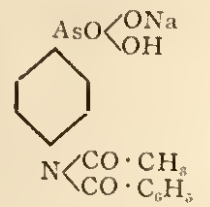
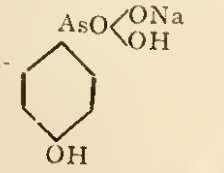
After a time, 20 c.c. of blood was taken from the jugular vein, and the serum used for analysis.

The arsenic was estimated in the same way as in our previous work; the slightly modified Sanger's method being adopted. Gold chloride was used as developer in preference to hydrochloric acid.

The results of the experiments are given in the following table

(Table I). For comparison, our previously recorded observations *in vitro* are appended:—

TABLE I

Drug	Chemical Constitution	Arsenic Estimation *in vivo*	Arsenic Estimation *in vitro*	Number of Experiments *in vivo*
Atoxyl		arsenic present	arsenic present	5
Sodium arsenate	—	arsenic* absent	arsenic absent	5
Acetylated Atoxyl		arsenic present	arsenic present	2
Benzoylated Atoxyl		arsenic present	arsenic present	2
Benzoyl-acetyl Atoxyl		arsenic present	arsenic absent	2
Sodium-p-hydroxyl-phenyl-arsenate		arsenic absent	arsenic absent	2

*In one of these experiments arsenic was found to be present. The fact that this particular serum contained haemoglobin may explain the exception.

Similar experiments were carried out on two donkeys in order to obtain larger quantities of blood, so as to make a more detailed examination of the distribution of the arsenic with regard to the constituents of the blood.

This table shows that the haemoglobin contained arsenic in both cases:—

TABLE II

	Atoxyl	Sodium Arsenate
Haemoglobin	arsenic present	arsenic present
Stroma	arsenic present	arsenic absent
Serum	arsenic present	arsenic absent

The above recorded experiments confirm and extend the view that the amido group in Atoxyl and allied compounds *in vitro*, as well as *in vivo*, combines with the serum proteins.

After the mode of the combination of Atoxyl and serum proteins in the animal organism had been established, it seemed necessary to estimate the amount of Atoxyl which is secreted in order to form an idea as to how much of the drug is actually left in the body.

For this purpose a horse was injected subcutaneously with Atoxyl, and the urine and faeces analysed. The arsenic was estimated according to Dupas-Gilier's[2] iodine method, using Gileas's and Shearer's modification.

Table III gives the amount of Atoxyl injected and recovered in urine and faeces:—

TABLE III

Urine

Date of Injection	Amount Injected	Date of Collecting	Arsenic Recovere
28.2.08	1 gm. Atoxyl	1.3.08	81%
2.3.08	1 gm. Atoxyl	3.3.08	83%
5.3.08	1 gm. Atoxyl	6.3.08	78%
7.3.08	1 gm. Atoxyl	8.3.08	82%
10.3.08	2 gm. Atoxyl	11.3.08	79%
12.3.08	2 gm. Atoxyl	13.3.08	85%
16.3.08	2 gm. Atoxyl	17.3.08	82%
19.3.08	2 gm. Atoxyl	20.3.08	80%
22.3.08	1 gm. Atoxyl	23.3.08	86%

Faeces

Date of Injection	Amount Injected	Date of Collecting	Arsenic Recovered
12.3.08	2 gm. Atoxyl	13.3.08	4%
16.3.08	2 gm. Atoxyl	17.3.08	2%
19.3.08	2 gm. Atoxyl	20.3.08	5%

The chemical details of this work will be published shortly.

Atoxyl has been found to be secreted in the urine, as:—

(1) p-amino-phenyl-arsenious acid;
(2) p-oxy-phenyl-arsenious acid;
(3) Arsyl-oxy-carbonyl.

The presence of this third compound in the urine is of practical interest, in so far as its formation can only be explained by assuming that Atoxyl is acetylated in the organism, and afterwards the p-amino-phenyl-arsenious acid is transformed into Arsyl-oxy-carbonyl. Similar observations[3] have been made on the secretion of p-toluidine, in which case Methyl-oxy-carbonyl is formed. It is quite possible that this may explain the fact that acetylated Atoxyl is less toxic than Atoxyl.

Besides arsenic, anilin was found to be present, *only* in the faeces. The faeces were treated with alkali, and steam passed through the mixture. The condensed steam gave distinctly, with bleaching powder, Hoffmann's reaction.

With regard to the distribution of Atoxyl in the organism, it has been shown by different authors that arsenic is deposited in all organs to a greater or lesser extent. J. Magalhães[4] states that Atoxyl does not permeate the meninges. We have attempted to verify his statement by using the cerebro-spinal fluid of infected donkeys withdrawn at different intervals after the last administration of the drug.

Table IV gives the results of the analysis:—

TABLE IV

Days elapsed since last Injection	Arsenic Examination
7 days	arsenic present
1 day	arsenic absent
9 days	arsenic absent
2 days	arsenic present
5 days	arsenic present
9 days	arsenic absent
3 days	arsenic present
6 days	arsenic present

These experiments prove conclusively that the meninges are permeable to Atoxyl, as in the majority of the recorded cases arsenic could be detected in the cerebro-spinal fluid.

LITERATURE

1. M. NIERENSTEIN. Comparative Chemo-therapeutical study of Atoxyl and Trypanocides, Part I. Annals of Tropical Medicine and Parasitology, Vol. II, No. 3, p. 249.

2. Zeitschrift für angewandte Chemie, Vol. 22, p. 378.

3. See HOPPE-SEYLER'S Zeitschrift für physiologische Chemie, Vol. 12, p. 295, also S. FRANKEL, Die Arzneimittel-Synthese auf Grundlage der Beziehungen zwischen chemischen Aufbau und Wirkung, 1906, p. 179

4. JOSÉ DE MAGALHÃES. Etude au point de vue therapeutique de la perméabilite meningée dans la Trypanosomiase humaine. XV Congrès International de Medicine, Lisbonne, 1906. Fascicule 2, p. 304; see also BREINL and TODD, British Medical Journal, 1907, January 19th, p. 132.